BIFUNCTIONAL ACYCLIC NUCLEOSIDE PHOSPHONATES: 2. SYMMETRICAL 2-{[BIS(PHOSPHONO)METHOXY]METHYL}ETHYL DERIVATIVES OF PURINES AND PYRIMIDINES

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Novel bisphosphonate alkylating agent, tetraisopropyl {2-[(mesyloxy)methyl]propane-1,3-diyl}bis(oxymethylene)bisphosphonate **19**, was synthesized from diethyl 2,2-bis-(hydroxymethyl)malonate. Decarbethoxylation of the diethyl 2,2-dimethyl-1,3-dioxane-5,5-dicarboxylate was followed by chloromethylation of 2-[(benzyloxy)methyl]propane-1,3-diol and Arbuzov reaction with triisopropyl phosphite. Bisphosphonate building block **19** was used in the alkylation of various nucleobases (2-amino-6-chloropurine, adenine, 2-amino-6-(cyclopropyl)aminopurine, cytosine, uracil and 4-methoxy-5-methylpyrimidin-2(1*H*)-one). N^9-Substituted purines and N^1-substituted pyrimidines were converted to appropriate free bisphosphonic acids. No antiviral or cytostatic activity was detected.

Keywords: Acyclic nucleoside phosphonates; ANPs; Acyclic nucleotide analogues; Phosphonomethyl ethers; Adefovir; Bisphosphonates; Nucleobases.

Analogues of nucleic acids components belong to antimetabolite group. Their ability to interfere with the metabolic pathways of nucleic acids synthesis de novo may cause antiviral, antineoplastic or other biological activity. First generation of nucleic acid analogues aimed at their close structural similarity to natural metabolites[1–3], however, led to their limited stability in the organism due to their easy degradation in the metabolic pathways.

The second generation of antimetabolites, acyclic nucleosides, has overcome this limitation by replacing the sugar moiety with aliphatic chain bound to the N^9 nitrogen of purines (e.g., acyclovir[4,5], ganciclovir[6,7], AHPA [8] or (*S*)-DHPA [9]). These compounds are in vivo phosphorylated to their 5′-nucleotide. However, the direct application of such nucleotides is not possible due to the lability of the phosphomonoester bond in blood plasma and during the transmembrane transport. Therefore, there is a need for new isopolar and isosteric phosphate analogues resistant to enzymatic reactions. One of the solutions was replacement of phosphate by enzymatically stable

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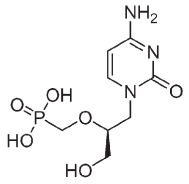
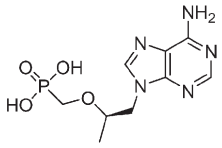
phosphonate group. Very interesting in this respect are phosphonomethyl ethers[10].

Structure–activity relationship investigations in the series of acyclic nucleotide analogues bearing a modified phosphoric acid residue in the side chain have revealed so far several biologically active acyclic nucleoside phosphonates (ANPs). Some of them are currently used in clinical practice[11,12]. Cidofovir or (*S*)-HPMPC **1** (Vistide®, an injectable form of Cidofovir) (Chart 1) is an antiviral medication for the treatment of cytomegalovirus (CMV) retinitis in patients with AIDS[13]. Tenofovir or (*R*)-PMPA **2**, and its prodrug Viread®, belongs to nucleotide reverse transcriptase inhibitors (NtRTIs) which block an enzyme crucial to viral production in HIV-infected people[14]. Adefovir or PMEA **3**, and its prodrug Hepsera®, is an orally-administered NtRTI used for treatment of hepatitis B[15].



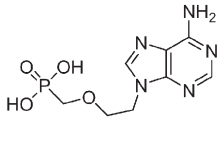
(*S*)-HPMPC (Cidofovir)

1



(*R*)-PMPA (Tenofovir)

2



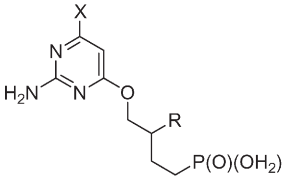
PMEA (Adefovir)

3

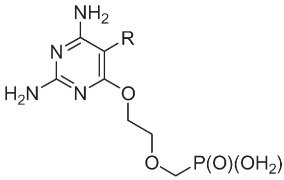
CHART 1

First generation of acyclic nucleoside phosphonates

Recently, attention was turned to the synthesis of a new type of ANPs originating from 2-substituted 4-amino-6-hydroxypyrimidines[16]. In these investigations, a significant potential activity of 6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives, compounds derived from 2,4-diaminopyrimidine (**4a**, **4c**, **4d**)[17] and 2-amino-4-hydroxypyrimidine (**4b**)[17], and their C5-substituted congeners **5** [18a,18b] was discovered (Chart 2).



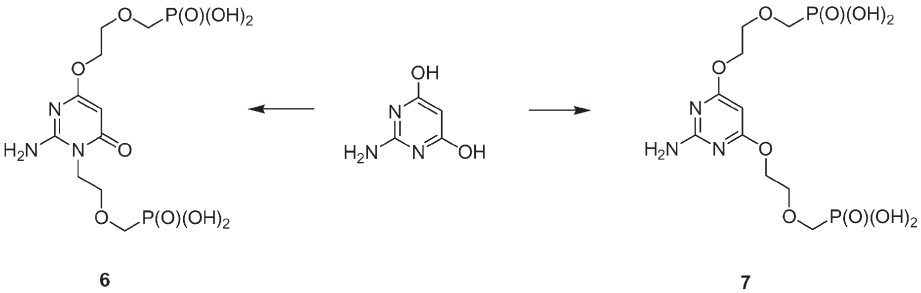
4a X= NH_2, R = H
4b X= OH, R = H
4c X = NH_2, R = (*R*)-CH_3
4d X = NH_2, R = (*S*)-CH_2OH



5 R = allyl, benzyl, CH_2CN, CH_2COOEt, CH_3, phenyl, cyclopropyl, CH_2COOH, Cl, Br, CN

CHART 2

Among the products isolated in these studies bisphosphonates **6** and **7** were also identified (Scheme 1)[16]. Despite the fact that these compounds constitute a new class of possible antiviral agents, they have not yet received much attention. The aim of this work was to explore the potential biological activity of such substances, which could be also considered as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor[19].



SCHEME 1

RESULTS AND DISCUSSION

In the previous paper[20] of this series we have described the synthesis of symmetrical 1,3-bis(phosphonomethoxy)propan-2-yl compounds **8**, derived from the 2-(phosphonomethoxy)ethyl (PME) chain, where an additional (phosphonomethoxy)methyl group is attached at the C1 position of the ethyl chain of the parent PME compound **9** (Chart 3).

In this paper we discuss the synthesis of another symmetrical acyclic nucleoside bisphosphonate (ANbP) congener **10** with the bisphosphonate chain extended by one carbon atom (Chart 3).

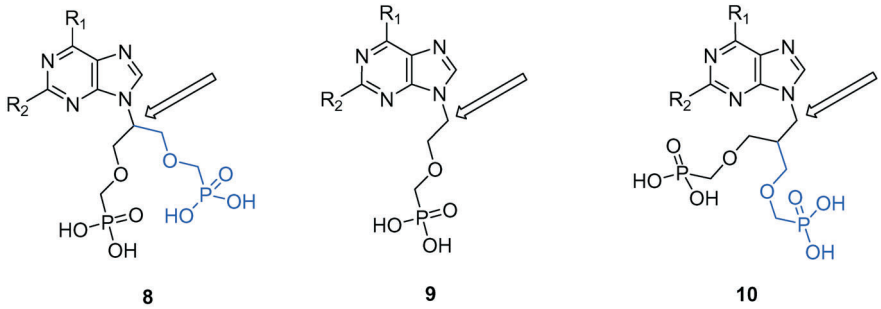
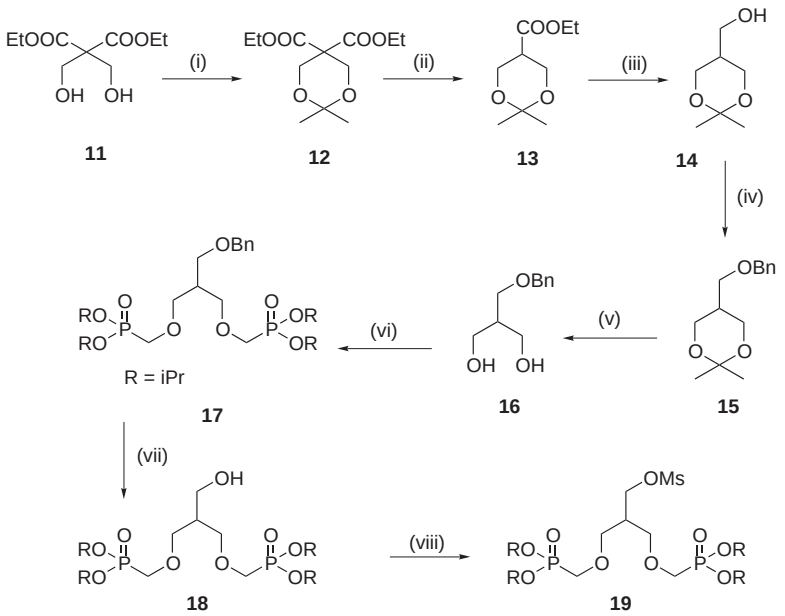


CHART 3

The strategy chosen for the synthesis of ANbP **10**, was based on alkylation of an appropriate heterocyclic base with reagent **19** which contains a good leaving group on an alkyl chain bearing an esterified bisphosphonate grouping linked through an ether bridge. This approach in most cases does not require any protection of the nucleobase and can be also applied to sensitive heterocyclic systems bearing reactive substituents (e.g., 6-chloropurines). In some cases, regioselectivity of the alkylation is limited and the separation of the formed isomers is inevitable.

Preparation of Bisphosphonate Alkylating Agent for Nucleobases (Scheme 2)

As shown in Scheme 2, the synthesis of the corresponding bisphosphonate alkylating agent **19** started from commercially available diethyl 2,2-bis-(hydroxymethyl)malonate **11**, which was transformed to an acetonide



(i) 2,2-dimethoxypropane, H_2SO_4, acetone; (ii) H_2O, NaCl, DMSO, 190-195 °C (65%); (iii) $LiAlH_4$, ether, 0 °C (99%); (iv) BnBr, NaH, THF; (v) Dowex 50 (H^+ form), 80% methanol, reflux (80%); (vi) $(CH_2O)_n$, HCl (g), $CaCl_2$, DCM, 0 °C, then $P(OiPr)_3$, 120 °C (85%); (vii) H_2/Pd/C, conc. HCl, MeOH (95%); (viii) MsCl, Et_3N, DCM (98%).

SCHEME 2

12 [21]. The mono ester **13** was obtained by decarbethoxylation of **12** in the presence of NaCl and DMSO according to the procedure described by Krapcho et al.[22–24] Ester **13** was reduced to primary alcohol **14** with LAH in ether and benzylated with benzyl bromide to afford benzyloxy derivative **15** which was used in the subsequent reaction without further purification. Acid hydrolysis of the isopropylidene protecting group of **15** gave 2-[(benzyloxy)methyl]propane-1,3-diol **16**. Chloromethylation of **16** with paraformaldehyde and gaseous HCl in dichloromethane followed by the Arbuzov reaction with triisopropyl phosphite gave the bisphosphonate **17**. The target bisphosphonate alkylating agent **19** was then prepared by hydrogenolysis of **17** followed by mesylation of **18** with methanesulfonyl chloride in anhydrous CH_2Cl_2 in the presence of Et_3N.

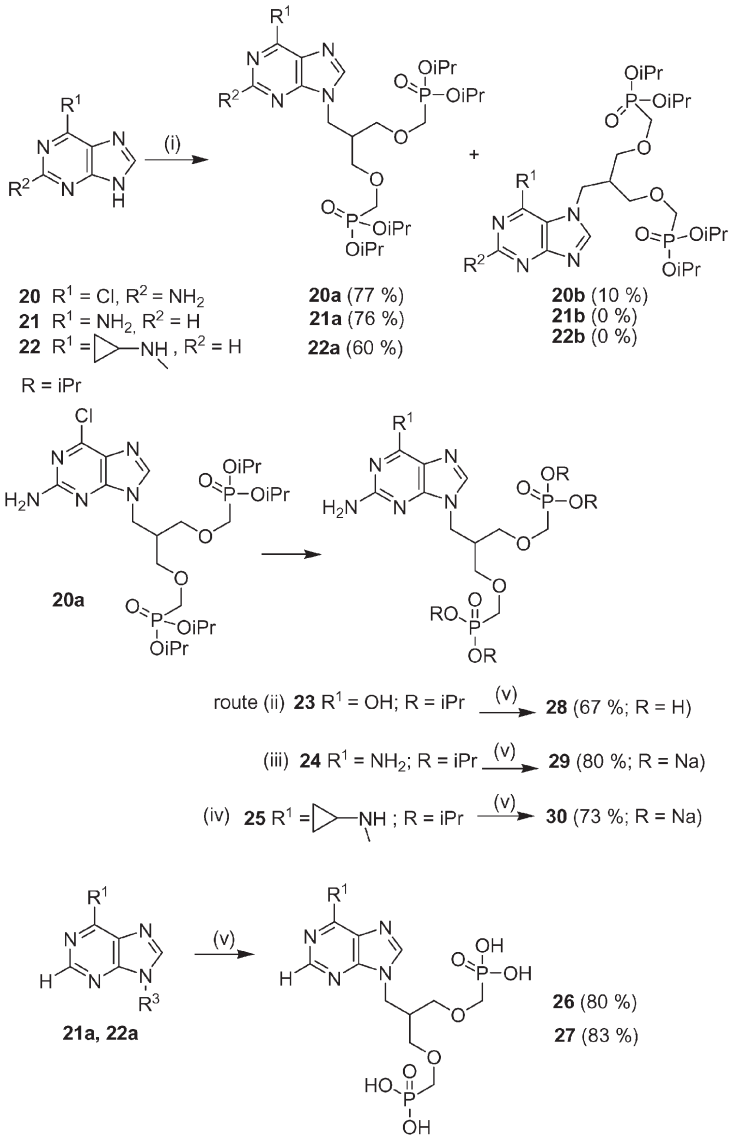
Alkylation of Nucleobases in General

The bisphosphonate **19** was used in alkylations of various nucleobases – 2-amino-6-chloropurine **20**, adenine **21**, 6-(cyclopropyl)aminopurine **22**, cytosine **31**, uracil **33**, 4-methoxy-5-methylpyrimidin-2(1*H*)-one **35**. All reactions were performed at 100 °C in the presence of Cs_2CO_3 in DMF and $CaCl_2$ protecting tube.

Alkylation of Purines (Scheme 3)

Alkylation of purines is depicted in Scheme 3. The reaction of 2-amino-6-chloropurine **20** easily proceeded at N^9 and also at N^7 positions to give regioisomers **20a** and **20b**. These isomers were easily separated by silica gel column chromatography. The intermediate **20a** was further converted to other base-modified bisphosphonates: thus, acid hydrolysis led to the guanine derivative **23**; the 2,6-diaminopurine derivative **24** was prepared by ammonolysis with methanolic ammonia, and replacement of chlorine with the cyclopropylamino group in the reaction with cyclopropylamine in dioxane gave 2-amino-6-(cyclopropyl)amino derivative **25**.

The alkylation of adenine **21** and 6-(cyclopropyl)aminopurine **22** gave exclusively the N^9-isomers **21a** and **22a**. In contrast to the analogous alkylation of **20**, essentially no N^7-regioisomer formation was observed in alkylation of compounds **21** and **22**. In all the discussed cases, NMR analysis was used to identify the position of substitution of the purine ring. All signals of hydrogen and carbon atoms were assigned using 2D-^{1}H,^{13}C HSQC and 2D-^{1}H,^{13}C HMBC experiments. In the case of N^9-isomers, protons on carbon atom next to the purine nitrogen have strong crosspeaks in HMBC



(i) **19**, Cs_2CO_3, DMF, 100 °C; (ii) 80% CH_3COOH, reflux (63 %); (iii) methanolic ammonia, MeOH, 100 °C, autoclave (65 %); (iv) cyclopropylamine, dioxane, reflux (84 %); (v) TMSBr, CH_3CN, RT.

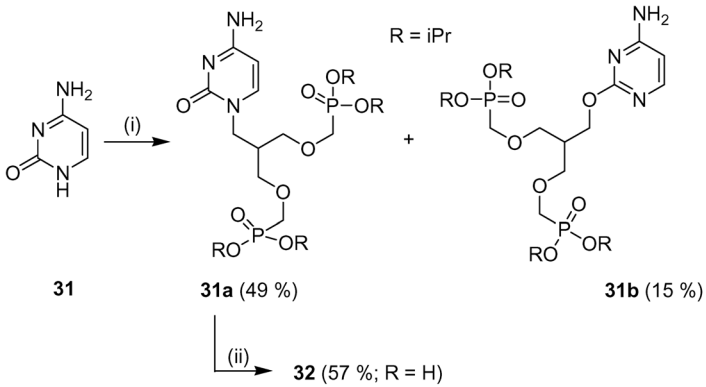
SCHEME 3

spectra with carbons C-4 and C-8 of the purine ring. In N^7-isomers these protons correlate with C-5 and C-8 atoms.

Subsequent deprotection of compounds **21a**, **22a**, **23** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free bisphosphonic acids **26**–**28**, which were ultimately purified by ion exchange chromatography. The behavior of compounds **24** and **25** differed from the above-mentioned substances. The deprotected and hydrolyzed products were applied onto Dowex 50X8 (H^+ form) as well. However, they adsorbed on the stationary phase and had to be eluted with dilute ammonia solution. Therefore, the obtained ammonia salts were finally transformed into sodium salts of bisphosphonic acids **29** and **30** by Dowex 50X8 (Na^+ form) chromatography.

Alkylation of Cytosine (Scheme 4)

The reaction of cytosine **31** with bisphosphonate **19** under the above conditions afforded a mixture of N^1-regioisomer (**31a**; yield 49%) and O^2-regioisomer (**31b**; yield 15%). Only the N^1 derivative was further converted to the corresponding free bisphosphonic acid **32** as shown in Scheme 4. O^2- and N^1-isomers in the pyrimidine series could be readily distinguished by NMR spectroscopy. The carbon atom chain linked to the oxygen shows δ 64 ppm; when bonded to nitrogen its chemical shift was δ 48 ppm (cf. Experimental). Furthermore, the protons bonded to this carbon have crosspeaks in 2D-^{1}H,^{13}C HMBC spectra to carbon atoms C-2 and C-6 (in the case of N^1-isomers) or only to C-2 (in the case of O^2-isomer).

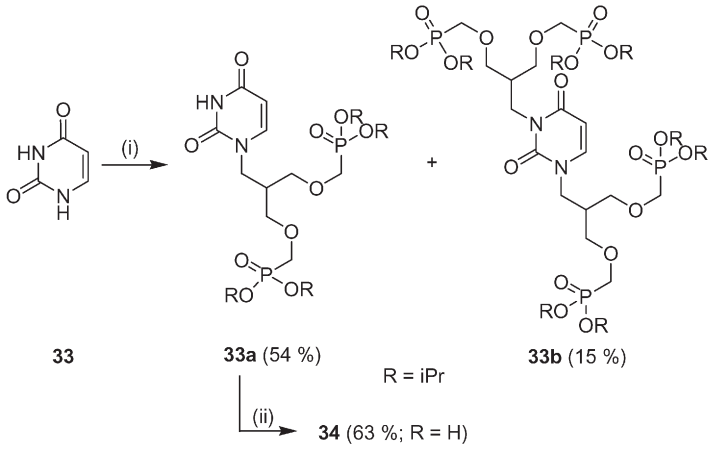


(i) **19**, Cs_2CO_3, DMF, 100 °C; (ii) TMSBr, CH_3CN, RT

SCHEME 4

Alkylation of Uracil (Scheme 5)

Scheme 5 shows the course of alkylation of uracil **33** with compound **19** under standard conditions. Of the two products formed in the reaction, N^1-alkylated **33a** and N^1,N^3-bisalkylated **33b**, the desired **33a** was the major product. It was isolated in about 54% yield while the bisalkylate was obtained in 15% yield. Subsequent deprotection of the tetraester **33a** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free bisphosphonic acid **34** which was isolated from the deionized product by ion exchange chromatography. The structure of all products was identified using NMR spectroscopy. In ^{1}H NMR spectra of the bisalkylated **33b** the NH signal is missing. In ^{13}C NMR, there are two signals of carbons bonded to the pyrimidine ring with δ 48 and 40 ppm for N^1 and N^3, respectively. Full assignment of all carbon and hydrogen atoms was done using 2D heterocorrelated experiments.



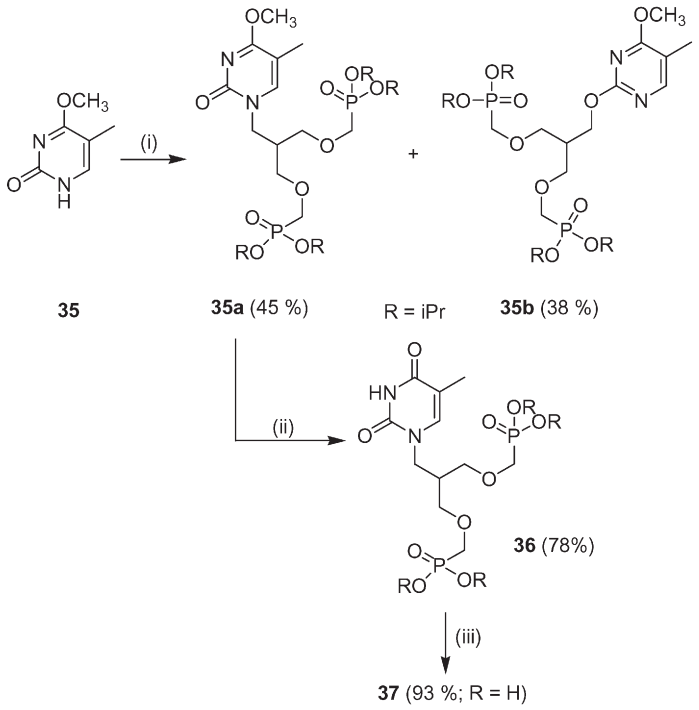
(i) **19**, Cs_2CO_3, DMF, 100 °C; (ii) TMSBr, CH_3CN, RT

SCHEME 5

Alkylation of 4-Methoxy-5-methylpyrimidin-2(1H)-one (Scheme 6)

Analogously to cytosine, also the alkylation of 4-methoxy-5-methylpyrimidin-2(1*H*)-one **35**, the precursor of thymine, provided two products (N^1-alkylate **35a** and O^2-alkylate **35b**) in the ratio almost 1:1. The procedure is depicted in Scheme 6. Compound **35a** was further hydrolyzed under acid conditions to provide thymine derivative **36**. It is worth mentioning that

attempts to prepare thymine derivative of **35b** failed. No reaction was observed in the reaction with 80% CH_3COOH and Dowex 50 (H^+ form). Decomposition of the phosphonate was achieved under more drastic conditions (heating with 2 M HCl). The subsequent deprotection of tetraester **36** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free phosphonic acid **37**, which was isolated from the deionized product by ion exchange chromatography. Both isolated isomers **35a** and **35b** could again be distinguished by NMR spectroscopy. All signals of hydrogen and carbon atoms were assigned using 1D and 2D NMR experiments. The position of the substituent is clear from the chemical shift of the carbon atom bonded to the pyrimidine ring (δ 65 ppm for the O^2 derivative and 48 ppm for the N^1 derivative) and is confirmed also by 2D HMBC spectra.



(i) **19**, Cs_2CO_3, DMF, 100 °C; (ii) 80% CH_3COOH, 80 °C; (iii) TMSBr, CH_3CN, RT

SCHEME 6

CONCLUSIONS

A novel bisphosphonate building block, tetraisopropyl {2-[(mesyloxy)-methyl]propane-1,3-diyl}bis(oxymethylene)bisphosphonate, was synthesized using transformation of diethyl 2,2-bis(hydroxymethyl)malonate as starting compound. The obtained alkylating agent was further used in alkylation of various nucleobases in the presence of Cs_2CO_3. While the alkylation of 2-amino-6-chloropurine afforded a mixture of N^9- and N^7-substituted purine derivatives, N^9-substituted nucleobases were obtained in the reaction with adenine and 2-amino-6-(cyclopropyl)aminopurine. A mixture of N^1- and O^2-regioisomers were obtained in the alkylation of cytosine and 4-methoxy-5-methylpyrimidin-2(1*H*)-one. The alkylation of uracil afforded a mixture of N^1-mono- and N^1,N^3-bisalkylated products. The corresponding free bisphosphonic acids were obtained after the ester cleavage from N^9-substituted purine and N^1-substituted pyrimidine derivatives. None of the prepared compounds exhibited any antiviral activity against DNA and RNA viruses. The compounds showed poor, if any, cytostatic activity. However, as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor, they might possess this activity. This alternative is currently being investigated.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried over P_2O_5 at 2 kPa. Melting points were determined on a Büchi melting point apparatus and are uncorrected. NMR spectra were measured on FT NMR spectrometer Varian Unity 500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix) or using EI (electron energy 70 eV). UV spectra (λ in nm) were taken on a Beckman Coulter™, DU® 800 spectrophotometer. Elemental analyses were carried out on a Perkin–Elmer CHN Analyser 2400, Series II Sys (Perkin–Elmer, Norwolk, CT, U.S.A.). Chemicals were purchased from Sigma–Aldrich (Prague, Czech Republic). Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4Å). Acetone was dried over anhydrous $CuSO_4$. Diethyl ether was distilled from $LiAlH_4$.

Diethyl 2,2-Dimethyl-1,3-dioxane-5,5-dicarboxylate (**12**)

Synthetic strategy and analysis corresponds to ref.[21]

Ethyl 2,2-Dimethyl-1,3-dioxane-5-carboxylate (**13**)

Synthetic strategy and analysis corresponds to refs[22–24].

(2,2-Dimethyl-1,3-dioxan-5-yl)methanol (**14**)

$LiAlH_4$ (4.6 g, 120.9 mmol) and dry diethyl ether (250 ml) were introduced into a well-dried three-neck vessel with condenser. The reaction mixture was cooled to 0 °C. Subsequently, compound **13** (28.08 g, 149.2 mmol) was added dropwise in 150 ml of diethyl ether in argon atmosphere during 30 min. The reaction proceeded under gentle reflux. The mixture was stirred under these conditions for another 3 h and at room temperature overnight. The residue was cooled with ice bath and 4.7 ml of water was slowly added. After that, 4.7 ml of 15% NaOH and 14.1 ml of water were added. The solution was stirred until a white precipitate formed. The mixture was filtered through Celite. The solid was washed with 200 ml of diethyl ether (twice). The filtrate was evaporated and used without further purification. FAB MS: 147.1 (MH^+) (70). ^{1}H NMR (500 MHz, DMSO-d_6): 4.54 (t, 1 H, $J_{OH\text{-}CH2}$ = 5.2, OH); 3.82 (dd, 2 H, $J_{2a\text{-}3}$ = 4.4, J_{gem} = 11.8, H-2a); 3.61 (dd, 2 H, $J_{2b\text{-}3}$ = 7.2, J_{gem} = 11.8, H-2b); 3.49 (dd, 2 H, $J_{4\text{-}3}$ = 6.7, $J_{4\text{-}OH}$ = 5.2, H-4); 1.69 (m, 1 H, H-3); 1.30 and 1.29 (2 × s, 6 H, 2 × CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 97.32 (C-1); 61.05 (C-2); 59.81 (C-4); 36.68 (C-3); 24.97 and 23.33 (2 × CH_3).

5-[(Benzyloxy)methyl]-2,2-dimethyl-1,3-dioxane (**15**)

Compound **14** (19 g, 130 mmol)) was added at 0 °C under argon atmosphere to a suspension of NaH (7.28 g of 60% suspension in mineral oil prewashed with *n*-hexane; 182 mmol) in dry THF (350 ml). The reaction mixture was then cooled to –10 °C and benzyl bromide (28.9 g, 169 mmol) in THF (200 ml) was added dropwise during 1 h. The mixture was stirred at –10 °C for 30 min and at room temperature overnight, under argon. When the reaction was complete (TLC), methanolic ammonia (30 ml) was added. After stirring the solution for 1 h, the solvent was evaporated. The residue in chloroform (500 ml) was washed with water (500 ml). The organic layer was dried with anhydrous $MgSO_4$, filtered and evaporated to yield crude product **15**, which was used without further purification.

2-[(Benzyloxy)methyl]propane-1,3-diol (**16**)

A crude mixture of **15** (30.7 g, 130 mmol) in 80% methanol (300 ml) was refluxed with Dowex 50X8 in H^+ form (10 g) for 4 h. The resin was filtered off, the solution neutralized with aqueous ammonia and the solvent was evaporated. The crude product was purified by silica gel column chromatography, using the chloroform–methanol gradient 0–6%, to yield 20.4 g (80%) of pure **16** as colorless oil. FAB MS: 197.2 (MH^+) (80). ^{1}H NMR (500 MHz, $CDCl_3$): 7.36–7.25 (m, 5 H, Ph); 4.44 (s, 2 H, OCH_2Ph); 4.40 (t, 2 H, $J_{OH\text{-}3}$ = 5.2, OH); 3.44 (t, 4 H, $J_{3\text{-}OH} \sim J_{3\text{-}2}$ = 5.3, H-3); 3.43 (d, 2 H, $J_{1\text{-}2}$ = 6.1, H-1); 1.80 (h, 1 H, $J_{2\text{-}3} \sim J_{2\text{-}1}$ = 6.0, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 139.03; 128.46 (2 C); 127.57 (2 C); 127.54; 72.39 (OCH_2Ph); 68.79 (d, $J_{C\text{-}P}$ = 12.7, C-1); 59.73 (C-3); 44.59 (C-2).

Diisopropyl [2-(Benzyloxymethyl)propane-1,3-diyl]bis(oxy)bis(methylene)bisphosphonate (**17**)

A mixture of compound **16** (12.3 g, 62.7 mmol), paraformaldehyde (2.2 equiv.) and $CaCl_2$ (4 g) was saturated with gaseous HCl at 0 °C for 45 min. The reaction mixture was stirred at 0 °C for 2 h, then allowed to reach room temperature, evaporated and dried. The residue was used without further purification in the Arbuzov reaction with triisopropyl phosphite.

The crude mixture was stirred at 100 °C, $P(OiPr)_3$ (30 ml, 2.1 equiv.) was slowly added and stirred for 1 h, keeping the temperature at 130 °C. The excess of phosphite and isopropyl chloride was distilled off (oil bath 80 °C) and the residue was purified by column chromatography on silica gel, using the chloroform–methanol gradient 0–3%, to yield 25 g (73%) of pure **17** as yellowish oil. FAB MS: 553.3 (MH^+) (80). ^{1}H NMR (500 MHz, $CDCl_3$): 7.36–7.25 (m, 5 H, Ph); 4.74 (m, 4 H, CH_{iPr}); 4.48 (s, 2 H, OCH_2Ph); 3.69 (d, 4 H, $J_{CH\text{-}P}$ = 8.6, OCH_2P); 3.62 (d, 4 H, $J_{1\text{-}2}$ = 6.0, H-1); 3.53 (d, 2 H, $J_{3\text{-}2}$ = 6.0, H-3); 2.26 (m, 1 H, $J_{2\text{-}3} \sim J_{2\text{-}1}$ = 6.0, H-2); 1.32 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 138.37; 128.32 (2 C); 127.52; 127.48 (2 C); 73.15 (OCH_2Ph); 71.75 (d, $J_{C\text{-}P}$ = 12.7, C-1); 71.18 (m, CH_{iPr}); 66.79 (C-3); 66.04 (d, $J_{C\text{-}P}$ = 168.0, OCH_2P); 40.20 (C-2); 24.06 (m, CH_3).

Diisopropyl [2-(Hydroxymethyl)propane-1,3-diyl]bis(oxy)bis(methylene)bisphosphonate (**18**)

Palladium on activated charcoal (10% Pd, 0.2 g) and concentrated HCl (0.1 ml) were added to a solution of **17** (3.68 g, 6.66 mmol) in methanol (30 ml). The reaction mixture was hydrogenated at atmospheric pressure and room temperature overnight. The catalyst was filtered off through a Celite pad, the filtrate was neutralized with Et_3N and evaporated. The crude product was purified by column chromatography on silica gel, using the chloroform–methanol gradient 0–6%, to yield 2.95 g (96%) of pure **18** as yellowish oil. FAB MS: 463.4 (MH^+) (80). ^{1}H NMR (500 MHz, $CDCl_3$): 4.74 (dh, 4 H, $J_{CH\text{-}P}$ = 7.6, $J_{CH\text{-}CH3}$ = 6.2, CH_{iPr}); 3.68 (d, 2 H, $J_{3\text{-}2}$ = 5.6, H-3); 3.66 (d, 4 H, $J_{CH\text{-}P}$ = 8.6, OCH_2P); 3.64 (m, 4 H, H-1); 3.44 (bt, 1 H, $J_{OH\text{-}H3}$ = 6.5, OH); 2.13 (m, 1 H, H-2); 1.34 and 1.33 (2 × d, 24 H, $J_{CH3\text{-}CH}$ = 6.2 and 6.2, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 72.02 (d, $J_{C\text{-}P}$ = 9.8, C-1); 71.11 (m, CH_{iPr}); 65.91 (d, $J_{C\text{-}P}$ = 167.4, OCH_2P); 61.29 (C-3); 41.73 (C-2); 24.02 (m, CH_3).

Diisopropyl [2-(Mesyloxymethyl)propane-1,3-diyl]bis(oxy)bis(methylene)bisphosphonate (**19**)

A mixture of **18** (1.3 g, 2.8 mmol) and Et_3N (1.5 equiv.) in dry dichloromethane (30 ml) was stirred at 0 °C with a $CaCl_2$ protecting tube. Mesyl chloride (1.1 equiv.) was added. The mixture was stirred at 0 °C for 1 h and then kept overnight in refrigerator. The solution was diluted with ice water (300 ml) and the layers separated. The organic layer was dried with anhydrous $MgSO_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel, using the chloroform–methanol gradient 0–4%, to yield 1.5 g (99%) of pure **19** as yellowish oil. For $C_{19}H_{42}O_{11}P_2S$ (540.5) calculated: 42.22% C, 7.83% H, 11.46% P, 5.93% S; found: 42.25% C, 7.87% H, 11.56% P, 6.05% S. FAB MS: 541.3 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 4.74 (dh, 4 H, $J_{CH\text{-}P}$ = 7.6, $J_{CH\text{-}CH3}$ = 6.2, CH_{iPr}); 4.32 (d, 2 H, $J_{3\text{-}2}$ = 5.6, H-3); 3.71 (d, 4 H, $J_{CH\text{-}P}$ = 8.6, OCH_2P); 3.64 (m, 4 H, H-1); 3.04 (s, 3 H, Ms-CH_3); 2.39 (h, 1 H, $J_{2\text{-}3} \sim J_{2\text{-}1}$ = 6.0, H-2); 1.34 and 1.33 (2 × d, 24 H, $J_{CH3\text{-}CH}$ = 6.2 and 6.1, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 71.07 (d, $J_{C\text{-}P}$ = 6.6, CH_{iPr}); 70.35 (d, $J_{C\text{-}P}$ = 11.8, C-1); 67.59 (C-3); 66.14 (d, $J_{C\text{-}P}$ = 168.5, OCH_2P); 39.59 (C-2); 37.00 (Ms-CH_3); 24.04 (m, CH_3).

Alkylation of Nucleobases with Compound **19**. General Procedure

A mixture of an appropriate nucleobase (**20**, **21**, **22**, **31**, **33**, **35**; 1 equiv.) and Cs_2CO_3 (0.5 equiv.) in dry DMF was stirred at room temperature for 1 h under a $CaCl_2$ protecting

tube. The reaction mixture was heated at 60 °C and bisphosphonate **19** (1.0 equiv.) was added. The mixture was then stirred at 100 °C for 24 h. The solvent was evaporated and the residue was co-evaporated with toluene. The residue dissolved in hot chloroform was filtered through a Celite pad, evaporated and purified on a silica gel column in chloroform–methanol.

2-Amino-6-chloro-9-(2-{[bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)purine (**20a**). Material: 2.8 mmol of **20**, 1.4 mmol of Cs_2CO_3, 2.8 mmol of **19**, 50 ml of DMF. Column chromatography (silica gel, 1–4% gradient of MeOH in $CHCl_3$) afforded the product **20a** as yellowish oil (yield 77%). FAB MS: 615.1 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 7.90 (s, 1 H, H-8); 5.46 (bs, 2 H, NH_2); 4.78 (m, 4 H, CH_{iPr}); 4.26 (d, 2 H, $J_{3',2'}$ = 7.5, H-3′); 3.72 (m, 4 H, OCH_2P); 3.50 (dd, 2 H, J_{gem} = 9.1 and $J_{1'a,2'}$ = 6.0, H-1′a); 3.48 (dd, 2 H, J_{gem} = 9.0 and $J_{1'b,2'}$ = 5.6, H-1′b); 2.52 (m, 1 H, H-2′); 1.36, 1.35, 1.34 and 1.33 (4 × d, 4 × 6 H, J_{CH3-CH} = 6.2, 6.2, 6.4 and 6.3, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.33 (C-2); 154.17 (C-4); 151.16 (C-6); 143.43 (C-8); 124.96 (C-5); 71.18 and 71.16 (2 × d, J_{C-P} = 6.6 and 6.7, CH_{iPr}); 70.94 (d, J_{C-P} = 12.9, C-1′); 66.11 (d, J_{C-P} = 169.3, OCH_2P); 41.46 (C-3′); 39.84 (C-2′); 24.08 (m, CH_3).

2-Amino-6-chloro-7-(2-{[bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)purine (**20b**). Material: 2.8 mmol of **20**, 1.4 mmol of Cs_2CO_3, 2.8 mmol of **19**, 50 ml of DMF. Column chromatography (silica gel, 1–6% gradient of MeOH in $CHCl_3$) afforded the product **20b** as yellowish oil (yield 10%). FAB MS: 615.1 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 8.18 (s, 1 H, H-8); 5.17 (bs, 2 H, NH_2); 4.76 (m, 4 H, CH_{iPr}); 4.46 (d, 2 H, $J_{3',2'}$ = 7.5, H-3′); 3.69 (m, 4 H, OCH_2P); 3.63 (dd, 2 H, J_{gem} = 9.5 and $J_{1'a,2'}$ = 5.2, H-1′a); 3.49 (dd, 2 H, J_{gem} = 9.5 and $J_{1'b,2'}$ = 5.0, H-1′b); 2.51 (m, 1 H, H-2′); 1.36, 1.35, 1.34 and 1.33 (4 × d, 4 × 6 H, J_{CH3-CH} = 6.2, 6.2, 6.4 and 6.3, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.38 (C-4); 159.33 (C-2); 150.03 (C-8); 143.29 (C-6); 116.17 (C-5); 71.15 (m, CH_{iPr}); 70.81 (d, J_{C-P} = 11.6, C-1′); 66.11 (d, J_{C-P} = 168.8, OCH_2P); 45.06 (C-3′); 41.06 (C-2′); 24.05 (m, CH_3).

9-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)adenine (**21a**). Material: 0.74 mmol of **21**, 0.37 mmol of Cs_2CO_3, 0.74 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1–8% gradient of MeOH in $CHCl_3$) afforded the product **21a** as yellowish oil (yield 76%). FAB MS: 580.5 (MH^+) (80). ^{1}H NMR (500 MHz, $CDCl_3$): 8.33 (s, 1 H, H-8); 8.00 (s, 1 H, H-2); 5.82 (bs, 2 H, NH_2); 4.76 (m, 4 H, CH_{iPr}); 4.32 (d, 2 H, $J_{3',2'}$ = 6.7, H-3′); 3.71 (m, 4 H, OCH_2P); 3.58 (dd, 2 H, J_{gem} = 9.6 and $J_{1'a,2'}$ = 5.8, H-1′a); 3.52 (dd, 2 H, J_{gem} = 9.6 and $J_{1'b,2'}$ = 5.6, H-1′b); 2.60 (m, 1 H, H-2′); 1.34 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.37 (C-6); 152.91 (C-2); 150.33 (C-4); 141.87 (C-8); 119.53 (C-5); 71.10 (m, CH_{iPr} and C-1′); 66.08 (d, J_{C-P} = 168.5, OCH_2P); 41.95 (C-3′); 39.90 (C-2′); 24.07 (m, CH_3).

6-(Cyclopropyl)amino-9-(2-{[bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)purine (**22a**). Material: 0.74 mmol of **22**, 0.37 mmol of Cs_2CO_3, 0.74 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1–5% gradient of MeOH in $CHCl_3$) afforded the product **22a** as yellowish oil (yield 60%). FAB MS: 620.6 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 8.45 (s, 1 H, H-2); 7.91 (s, 1 H, H-8); 5.99 (bs, 1 H, NH); 4.76 (m, 4 H, CH_{iPr}); 4.31 (d, 2 H, $J_{3',2'}$ = 6.7, H-3′); 3.70 (d, 4 H, J_{H-C-P} = 8.6, OCH_2P); 3.57 (dd, 2 H, J_{gem} = 9.5 and $J_{1'a,2'}$ = 5.8, H-1′a); 3.51 (dd, 2 H, J_{gem} = 9.5 and $J_{1'b,2'}$ = 5.7, H-1′b); 3.04 (m, 1 H, CH_{cycl}); 2.59 (m, 1 H, H-2′); 1.35 (d, 12 H, $J_{CH3,CH}$ = 6.1, CH_3); 1.33 (d, 12 H, $J_{CH3,CH}$ = 6.1, CH_3); 0.93 (m, 2 H, CH_{2cycl}); 0.67 (m, 2 H, CH_{2cycl}). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.72 (C-6); 153.16 (C-2); 149.22 (C-4); 141.21 (C-8); 119.81 (C-5); 71.10 (m, C-1′ and CH_{iPr}); 66.07 (d, J_{C-P} = 168.4, OCH_2P); 41.87 (C-3′); 39.94 (C-2′); 24.07 (m, CH_3).

1-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)cytosine (**31a**). Material: 0.87 mmol of **31**, 0.43 mmol of Cs_2CO_3, 0.87 mmol of **19**, 40 ml of DMF. Column chromatography (silica gel, 1–15% gradient of MeOH in $CHCl_3$) afforded the product **31a** as yellowish oil (yield

49%). FAB MS: 556.4 (MH^+) (45). ^{1}H NMR (500 MHz, $CDCl_3$): 7.51 (d, 1 H, $J_{H\text{-}6,H\text{-}5}$ = 7.2, H-6); 5.75 (d, 1 H, $J_{H\text{-}5,H\text{-}6}$ = 7.2, H-5); 4.74 (m, 4 H, CH_{iPr}); 3.81 (d, 2 H, $J_{3',2'}$ = 6.8, H-3′); 3.68 (m, 4 H, OCH_2P); 3.60 (dd, 2 H, J_{gem} = 9.6 and $J_{1'a,2'}$ = 5.3, H-1′a); 3.54 (dd, 2 H, J_{gem} = 9.6 and $J_{1'b,2'}$ = 5.8, H-1′b); 2.52 (m, 1 H, H-2′); 1.33 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 165.87 (C-4); 155.75 (C-2); 147.26 (C-6); 93.78 (C-5); 71.32 (d, $J_{1',P}$ = 12.9, C-1′); 71.06 (m, CH_{iPr}); 65.93 (d, $J_{C\text{-}P}$ = 169.0, OCH_2P); 48.60 (C-3′); 38.63 (C-2′); 24.07 (m, CH_3).

2-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)cytosine (**31b**). Material: 0.87 mmol of **31**, 0.43 mmol of Cs_2CO_3, 0.87 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1–7% gradient of MeOH in $CHCl_3$) afforded the product **31b** as yellowish oil (yield 15%). FAB MS: 556.5 (MH^+) (45). ^{1}H NMR (500 MHz, $CDCl_3$): 7.96 (d, 1 H, $J_{H\text{-}6,H\text{-}5}$ = 5.5, H-6); 6.11 (d, 1 H, $J_{H\text{-}5,H\text{-}6}$ = 5.7, H-5); 5.64 (bs, 1 H, NH_2); 4.74 (m, 4 H, CH_{iPr}); 4.34 (d, 2 H, $J_{3',2'}$ = 6.8, H-3′); 3.76–3.68 (m, 8H, H-1′ and OCH_2P); 2.44 (m, 1 H, H-2′); 1.32 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.83 and 164.78 (C-4 and C-2); 156.90 (C-6); 99.19 (C-5); 71.13 (d, $J_{1',P}$ = 12.6, C-1′); 70.80 (d, $J_{CH\text{-}P}$ = 6.6, CH_{iPr}); 65.79 (d, $J_{C\text{-}P}$ = 168.2, OCH_2P); 64.19 (C-3′); 39.18 (C-2′); 23.82 (d, $J_{C\text{-}P}$ = 3.7, CH_3); 23.72 (d, $J_{C\text{-}P}$ = 4.5, CH_3).

1-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)uracil (**33a**). Material: 0.87 mmol of **33**, 0.43 mmol of Cs_2CO_3, 0.87 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1–5% gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **33a** and **33b**. Compound **33a** was separated from **33b** on thin layer chromatography in 10% MeOH/$CHCl_3$ as yellowish oil (54%). FAB MS: 557.5 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 8.72 (bs, 1 H, NH); 7.51 (d, 1 H, $J_{H\text{-}6,H\text{-}5}$ = 7.9, H-6); 5.68 (dd, 1 H, $J_{H\text{-}5,H\text{-}6}$ = 7.9, $J_{H\text{-}5,3'}$ = 2.3, H-5); 4.75 (m, 4 H, CH_{iPr}); 3.82 (d, 2 H, $J_{3',2'}$ = 6.7, H-3′); 3.69 (m, 4 H, OCH_2P); 3.61 (dd, 2 H, J_{gem} = 9.6 and $J_{1'a,2'}$ = 5.6, H-1′a); 3.57 (dd, 2 H, J_{gem} = 9.6 and $J_{1'b,2'}$ = 5.5, H-1′b); 2.42 (m, 1 H, H-2′); 1.34 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 163.55 (C-4); 150.88 (C-2); 146.17 (C-6); 101.68 (C-5); 71.26 (d, $J_{1',P}$ = 12.2, C-1′); 71.10 (m, CH_{iPr}); 66.04 (d, $J_{C\text{-}P}$ = 169.1, OCH_2P); 47.62 (C-3′); 38.98 (C-2′); 24.06 (m, CH_3).

1,3-[Bis(2,2′-{[bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)]uracil (**33b**). Material: 0.87 mmol of **33**, 0.43 mmol of Cs_2CO_3, 0.87 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1–5% gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **33a** and **33b**. Compound **33b** was separated from **33a** on thin layer chromatography in 10% MeOH/$CHCl_3$ as yellowish oil (15%). FAB MS: 1001.9 (MH^+) (85). ^{1}H NMR (500 MHz, $CDCl_3$): 7.45 (d, 1 H, $J_{H\text{-}6,H\text{-}5}$ = 7.9, H-6); 5.68 (d, 1 H, $J_{H\text{-}5,H\text{-}6}$ = 7.9, H-5); 4.74 (m, 8 H, CH_{iPr}); 4.00 (d, 2 H, $J_{3'',2''}$ = 6.9, H-3″); 3.80 (d, 2 H, $J_{3',2'}$ = 6.8, H-3′); 3.75–3.53 (m, 16 H, H-1′, H-1″, OCH_2P, OCH_2P'); 2.39 (m, 2 H, H-2′ and H-2″); 1.35–1.31 (m, 48 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 163.32 (C-4); 151.72 (C-2); 144.06 (C-6); 100.99 (C-5); 72.38 (d, $J_{1'',P}$ = 12.8, C-1″); 71.29–70.85 (m, H-1′ and CH_{iPr}); 66.08 and 66.04 (d, $J_{C\text{-}P}$ = 167.8 and 168.9, OCH_2P and OCH_2P'); 48.58 (C-3′); 40.77 (C-3″); 38.85 (C-2′); 38.51 (C-2″); 24.15–24.00 (m, CH_3).

1-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)-4-methoxythymine (**35a**). Material: 1.85 mmol of **35**, 0.92 mmol of Cs_2CO_3, 1.85 mmol of **19**, 25 ml of DMF. Column chromatography (silica gel, 1–3% gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **35a** and **35b**. Compound **35a** was separated from **35b** on thin layer chromatography in 5% MeOH/$CHCl_3$ as yellowish oil (45%). FAB MS: 585.5 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 7.51 (q, 1 H, $J_{H\text{-}6,CH3}$ = 1.1, H-6); 4.74 (m, 4 H, CH_{iPr}); 3.97 (s, 3 H, OCH_3); 3.87 (d, $J_{3',2'}$ = 6.9, H-3′); 3.68 (m, 4 H, OCH_2P); 3.60 (dd, 2 H, J_{gem} = 9.5 and $J_{1'a,2'}$ = 5.2, H-1′a); 3.52 (dd, 2 H, J_{gem} = 9.5 and $J_{1'b,2'}$ = 6.0, H-1′b); 2.53 (m, 1 H, H-2′); 1.96 (d, 3 H, $J_{CH3,H\text{-}6}$ = 1.1, Py-CH_3); 1.35–1.31 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 170.79 (C-4); 156.88

(C-2); 145.64 (C-6); 104.29 (C-5); 71.24–70.96 (m, C-1′ and CH_{iPr}); 65.97 (d, J_{C-P} = 169.1, OCH_2P); 54.44 (OCH_3); 48.43 (C-3′); 38.61 (C-2′); 24.07 (m, CH_3); 11.80 (Py-CH_3).

2-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)-4-methoxythymine (**35b**). Material: 1.85 mmol of **35**, 0.92 mmol of Cs_2CO_3, 1.85 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1–3% gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **35a** and **35b**. Compound **35b** was separated from **35a** on thin layer chromatography in 5% MeOH/$CHCl_3$ as yellowish oil (38%). FAB MS: 585.6 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 7.95 (s, 1 H, H-6); 4.73 (m, 4 H, CH_{iPr}); 4.37 (d, 2 H, $J_{3',2'}$ = 6.2, H-3′); 3.98 (s, 3 H, OCH_3); 3.74–3.70 (m, 8 H, H-1′ and OCH_2P); 2.49 (m, 1 H, H-2′); 2.05 (d, 3 H, $J_{CH3,H-6}$ = 0.7, Py-CH_3); 1.31 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 169.61 (C-4); 163.60 (C-2); 157.00 (C-6); 111.10 (C-5); 71.40 (d, $J_{1',P}$ = 12.7, C-1′); 70.97 (d, $J_{CH,P}$ = 6.3, CH_{iPr}); 66.10 (d, J_{C-P} = 168.1, OCH_2P); 65.14 (C-3′); 53.86 (OCH_3); 39.42 (C-2′); 24.04 (m, CH_3); 11.84 (Py-CH_3).

9-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)guanine (**23**)

A solution of **20a** (370 mg, 0.6 mmol) in 80% acetic acid (20 ml) was refluxed for 6 h. The solution was neutralized with Et_3N and the volatiles were evaporated in vacuo. Column chromatography (silica gel, 1–15% gradient of MeOH in $CHCl_3$) afforded compound **23** as a white amorphous powder (yield 63%). FAB MS: 596.3 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 11.43 (bs, 1 H, NH); 7.58 (s, 1 H, H-8); 7.02 (bs, 2 H, NH_2); 4.80 (m, 4 H, CH_{iPr}); 4.09 (d, 2 H, $J_{3',2'}$ = 7.6, H-3′); 3.69 (m, 4 H, OCH_2P); 3.56 (dd, 2 H, J_{gem} = 9.1 and $J_{1'a,2'}$ = 6.0, H-1′a); 3.47 (dd, 2 H, J_{gem} = 9.0 and $J_{1'b,2'}$ = 4.4, H-1′b); 2.49 (m, 1 H, H-2′); 1.35 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 157.89 (C-6); 154.46 (C-2); 150.82 (C-4); 138.23 (C-8); 117.60 (C-5); 71.61 (m, CH_{iPr} and C-1′); 66.10 (d, J_{C-P} = 170.7, OCH_2P); 42.01 (C-3′); 39.42 (C-2′); 24.05 (m, CH_3).

2,6-Diamino-9-(2-{[bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)purine (**24**)

A solution of **20a** (570 mg, 0.93 mmol) in methanolic ammonia (50 ml) was heated (100 °C) in an autoclave for 13 h. The solvent was then evaporated. Purification of the residue by column chromatography (silica gel, 1–12% gradient of MeOH in $CHCl_3$) afforded compound **24** as yellowish foam (yield 65%). FAB MS: 595.3 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 7.63 (s, 1 H, H-8); 5.52 (bs, 2 H, 6-NH_2); 4.91 (bs, 2 H, 2-NH_2); 4.77 (m, 4 H, CH_{iPr}); 4.16 (d, 2 H, $J_{3',2'}$ = 6.5, H-3′); 3.76 and 3.70 (2 × dd, 4 H, J_{gem} = 13.5 and 13.5, J_{H-C-P} = 8.9 and 8.8, OCH_2P); 3.54 (dd, 2 H, J_{gem} = 9.5 and $J_{1'a,2'}$ = 6.1, H-1′a); 3.49 (dd, 2 H, J_{gem} = 9.6 and $J_{1'b,2'}$ = 5.4, H-1′b); 2.53 (m, 1 H, H-2′); 1.35 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.90 (C-2); 155.73 (C-6); 152.41 (C-4); 139.16 (C-8); 114.20 (C-5); 71.07 (m, CH_{iPr} and C-1′); 66.03 (d, J_{C-P} = 168.8, OCH_2P); 41.16 (C-3′); 39.77 (C-2′); 24.08 (m, CH_3).

2-Amino-6-(cyclopropyl)amino-9-(2-{[bis(diisopropyloxyphosphoryl)methoxy]methyl}-ethyl)purine (**25**)

A solution of **20a** (430 mg, 0.7 mmol) and cyclopropylamine (9 equiv.) in dioxane (20 ml) was refluxed for 2 days. The solvent and excess of amine were evaporated to dryness. Purification of compound by column chromatography (silica gel, 1–6% gradient of MeOH in $CHCl_3$) afforded the product **25** as yellowish oil (yield 84%). FAB MS: 635.6 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 7.57 (s, 1 H, H-8); 5.81 (bs, 1 H, NH_{cycl}); 4.93 (bs, 2 H, NH_2); 4.77 (m, 4 H,

CH_{iPr}); 4.15 (d, 2 H, $J_{3',2'}$ = 6.6, H-3′); 3.75 and 3.70 (2 × dd, 4 H, J_{gem} = 13.5 and 13.5, J_{H-C-P} = 9.0 and 8.8, OCH_2P); 3.53 (dd, 2 H, J_{gem} = 9.6 and $J_{1'a,2'}$ = 6.1, H-1′a); 3.48 (dd, 2 H, J_{gem} = 9.6 and $J_{1'b,2'}$ = 5.4, H-1′b); 2.99 (bs, 1 H, CH_{cycl}); 2.52 (m, 1 H, H-2′); 1.34 (m, 24 H, CH_3); 0.85 (m, 2 H, CH_{2cycl}); 0.61 (m, 2 H, CH_{2cycl}). ^{13}C NMR (125.7 MHz, $CDCl_3$): 160.07 (C-2); 156.17 (C-6); 151.36 (C-4); 138.38 (C-8); 114.38 (C-5); 71.07 (m, CH_{iPr} and C-1′); 66.01 (d, J_{C-P} = 168.9, OCH_2P); 41.08 (C-3′); 39.80 (C-2′); 24.07 (m, CH_3); 23.60 (CH_{cycl}); 7.37 (CH_{2cycl}).

1-(2-{[Bis(diisopropyloxyphosphoryl)methoxy]methyl}ethyl)thymine (**36**)

A solution of **35a** (480 mg, 0.8 mmol) in 80% acetic acid (15 ml) was refluxed for 24 h. The solution was neutralized with Et_3N. Solvent and excess of acetic acid were evaporated. The purification of the residue by column chromatography (silica gel, 1–3% gradient of MeOH in $CHCl_3$) afforded the product **36** as yellowish oil (yield 78%). FAB MS: 571.2 (MH^+) (60). ^{1}H NMR (500 MHz, $CDCl_3$): 8.59 (s, 1 H, NH); 7.33 (d, 1 H, $J_{H-6,CH3}$ = 1.2, H-6); 4.75 (m, 4 H, CH_{iPr}); 3.79 (d, 2 H, $J_{3',2'}$ = 6.8, H-3′); 3.69 (m, 4 H, OCH_2P); 3.60 (dd, 2 H, J_{gem} = 9.5 and $J_{1'a,2'}$ = 5.5, H-1′a); 3.51 (dd, 2 H, J_{gem} = 9.5 and $J_{1'b,2'}$ = 5.6, H-1′b); 1.95 (d, 3 H, $J_{CH3,H-6}$ = 1.1, Py-CH_3); 1.34 (m, 24 H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.16 (C-4); 150.91 (C-2); 141.89 (C-6); 110.32 (C-5); 71.23 (d, $J_{1',P}$ = 12.7, C-1′); 71.07 (d, J_{C-O-P} = 7.0, CH_{iPr}); 71.03 (d, J_{C-O-P} = 6.9, CH_{iPr}); 66.01 (d, J_{C-P} = 169.2, OCH_2P); 47.19 (C-3′); 39.07 (C-2′); 24.05 (m, CH_3); 12.06 (Py-CH_3).

Transformation of Esters to Free Phosphonic Acids. General Procedure

The dried starting esters (**21a**, **22a**, **23**, **24**, **25**, **31a**, **33a** and **36**), acetonitrile (15 ml) and $BrSiMe_3$ (excess) were stirred at room temperature overnight. After evaporation and co-distillation with acetonitrile, the residue was treated with water and aqueous ammonia. The mixture was evaporated to dryness, the residue dissolved in water was applied onto a column of Dowex 50X8 in H^+ form and washed with water.

A) Elution with water and evaporation in vacuo afforded free phosphonic acids **26**–**28**, **32**, **34**, and **36**.

B) Elution with dilute ammonia and evaporation afforded ammonium salts, which were applied onto Dowex 50 (Na^+ form). Elution with water and evaporation gave phosphonic acids **29** and **30** as tetrasodium salts.

9-(2-{[Bis(phosphono)methoxy]methyl}ethyl)adenine (**26**). Material: 0.50 mmol of **21a**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 80%), m.p. 248.9 °C (dec.). For $C_{11}H_{19}N_5O_8P_2$ (411.2) calculated: 32.13% C, 4.66% H, 17.03% N, 15.06% P; found: 32.07% C, 4.76% H, 17.15% N, 15.13% P. FAB MS: 412.1 (MH^+) (45). ^{1}H NMR (500 MHz, D_2O + NaOD): 8.22 and 8.21 (s, 2 H, H-8 and H-2); 4.37 (d, 2 H, $J_{3',2'}$ = 6.7, H-3′); 3.58 (m, 8 H, H-1′ and OCH_2P); 2.56 (m, 1 H, H-2′). ^{13}C NMR (125.7 MHz, D_2O + NaOD): 156.10 (C-6); 152.93 (C-2); 149.79 (C-4); 144.04 (C-8); 119.03 (C-5); 71.50 (d, $J_{1',P}$ = 11.6, C-1′); 67.95 (d, J_{C-P} = 156.2, OCH_2P); 43.15 (C-3′); 39.85 (C-2′). UV: (0.01 M HCl) λ_{max} = 258 nm (ε_{max} = 13768); (H_2O) λ_{max} = 259 nm (ε_{max} = 14352); (0.01 M NaOH) λ_{max} = 259 nm (ε_{max} = 13402).

6-(Cyclopropyl)amino-9-(2-{[bis(phosphono)methoxy]methyl}ethyl)purine (**27**). Material: 0.39 mmol of **22a**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 80%), m.p. 205.0 °C (dec.). For $C_{14}H_{23}N_5O_8P_2$ (451.3) calculated: 37.26% C, 5.14% H, 15.52% N, 13.73% P; found: 37.37% C, 5.25% H, 15.68% N, 13.82% P. FAB MS: 452.3 (MH^+) (60). ^{1}H NMR (500 MHz,

D_2O): 8.49 (s, 1 H, H-2); 8.38 (s, 1 H, H-8); 4.49 (d, 2 H, $J_{3',2'}$ = 6.7, H-3′); 3.69–3.60 (m, 8 H, H-1′ and OCH_2P); 2.91 (bs, 1 H, CH_{cycl}); 2.62 (m, 1 H, H-2′); 1.11 (m, 2 H, CH_{2cycl}); 0.90 (m, 2 H, CH_{2cycl}). ^{13}C NMR (125.7 MHz, D_2O): 150.61 (C-6); 148.35 (C-4); 146.08 (C-8); 144.71 (C-2); 119.08 (C-5); 71.72 (d, $J_{1',P}$ = 11.8, C-1′); 67.24 (d, J_{C-P} = 158.2, OCH_2P); 44.04 (C-3′); 39.89 (C-2′); 23.42 (CH_{cycl}); 7.33 (CH_{2cycl}). UV: (0.01 M HCl) λ_{max} = 266 nm (ε_{max} = 18646); (H_2O) λ_{max} = 266 nm (ε_{max} = 18340); (0.01 M NaOH) λ_{max} = 268 nm (ε_{max} = 17518).

9-(2-{[Bis(phosphono)methoxy]methyl}ethyl)guanine (**28**). Material: 0.37 mmol of **23**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 67%), m.p. 206.9 °C (dec.). For $C_{11}H_{19}N_5O_9P_2$ (427.2) calculated: 30.92% C, 4.48% H, 16.39% N, 14.50% P; found: 30.85% C, 4.58% H, 16.50% N, 14.56% P. FAB MS: 428.3 (MH^+) (60). ^1H NMR (500 MHz, D_2O): 8.98 (s, 1 H, H-8); 4.44 (d, 2 H, $J_{3',2'}$ = 6.5, H-3′); 3.72–3.61 (m, 8 H, H-1′ and OCH_2P); 2.64 (m, 1 H, H-2′). ^{13}C NMR (125.7 MHz, D_2O): 156.02 and 155.97 (C-6 and C-2); 150.92 (C-4); 138.95 (C-8); 108.45 (C-5); 71.73 (d, $J_{1',P}$ = 12.4, C-1′); 67.28 (d, J_{C-P} = 158.1, OCH_2P); 45.17 (C-3′); 39.12 (C-2′). UV: (0.01 M HCl) λ_{max} = 252 nm (ε_{max} = 10558); (H_2O) λ_{max} = 251 nm (ε_{max} = 10890); (0.01 M NaOH) λ_{max} = 266 nm (ε_{max} = 9320).

Tetrasodium salt of 2,6-diamino-9-(2-{[bis(phosphono)methoxy]methyl}ethyl)purine (**29**). Material: 0.60 mmol of **24**, 3.5 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 88%), m.p. 327.1 °C (dec.). FAB MS: 515.0 (MH^+) (30). HR MS for $C_{11}H_{16}N_6Na_4O_8P_2$ calculated: 515.01546, found: 515.01739. ^1H NMR (500 MHz, D_2O): 7.91 (s, 1 H, H-8); 4.23 (d, 2 H, $J_{3',2'}$ = 6.8, H-3′); 3.95 (m, 8 H, H-1′ and OCH_2P); 2.51 (m, 1 H, H-2′). ^{13}C NMR (125.7 MHz, D_2O): 160.35 (C-6); 156.61 (C-2); 151.79 (C-4); 141.86 (C-8); 113.49 (C-5); 71.51 (d, $J_{1',P}$ = 11.8, C-1′); 68.56 (d, J_{C-P} = 154.5, OCH_2P); 42.62 (C-3′); 39.68 (C-2′). UV: (0.01 M HCl) λ_{max} = 284 nm (ε_{max} = 9092); (H_2O) λ_{max} = 278 nm (ε_{max} = 10120); (0.01 M NaOH) λ_{max} = 278 nm (ε_{max} = 9442).

Tetrasodium salt of 2-amino-6-(cyclopropyl)amino-9-(2-{[bis(phosphono)methoxy]methyl}ethyl)-purine (**30**). Material: 0.54 mmol of **25**, 3.5 ml of TMSBr, 15 ml of CH_3CN. Yellow solid (yield 73%), m.p. 338.8 °C (dec.). FAB MS: 555.0 (MH^+) (30). HR MS for $C_{14}H_{20}N_6Na_4O_8P_2$ calculated: 555.0460, found: 555.0487. ^1H NMR (500 MHz, D_2O): 7.89 (s, 1 H, H-8); 4.24 (d, 2 H, $J_{3',2'}$ = 6.7, H-3′); 3.54 (m, 8 H, H-1′ and OCH_2P); 2.86 (bs, 1 H, CH_{cycl}); 2.52 (m, 1 H, H-2′); 0.88 (m, 2 H, CH_{2cycl}); 0.67 (m, 2 H, CH_{2cycl}). ^{13}C NMR (125.7 MHz, D_2O): 160.59 (C-2); 156.99 (C-6); 150.75 (C-4); 141.39 (C-8); 113.85 (C-5); 71.52 (d, $J_{1',P}$ = 9.6, C-1′); 68.83 (d, J_{C-P} = 153.7, OCH_2P); 42.58 (C-3′); 39.69 (C-2′); 23.88 (CH_{cycl}); 7.37 (CH_{2cycl}). UV: (0.01 M HCl) λ_{max} = 282 nm (ε_{max} = 13712); (H_2O) λ_{max} = 282 nm (ε_{max} = 13736); (0.01 M NaOH) λ_{max} = 290 nm (ε_{max} = 12008).

1-(2-{[Bis(phosphono)methoxy]methyl}ethyl)cytosine (**32**). Material: 0.36 mmol of **31a**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 57%), m.p. 208.5 °C (dec.). For $C_{10}H_{19}N_3O_9P_2$ (387.2) calculated: 31.02% C, 4.95% H, 10.85% N, 16.00% P; found: 31.05% C, 4.85% H, 10.75% N, 16.15% P. FAB MS: 388.3 (MH^+) (30). ^1H NMR (500 MHz, D_2O): 7.93 (d, 1 H, $J_{H-6,H-5}$ = 7.7, H-6); 6.17 (d, 1 H, $J_{H-5,H-6}$ = 7.7, H-5); 4.00 (d, 2 H, $J_{3',2'}$ = 6.9, H-3′); 3.65 (m, 8 H, H-1′ and OCH_2P); 2.47 (m, 1 H, H-2′). ^{13}C NMR (125.7 MHz, D_2O): 160.19 (C-4); 151.21 (C-6); 149.99 (C-2); 94.92 (C-5); 71.91 (d, $J_{1',P}$ = 12.1, C-1′); 67.27 (d, J_{C-P} = 158.1, OCH_2P); 50.03 (C-3′); 38.50 (C-2′). UV: (0.01 M HCl) λ_{max} = 281 nm (ε_{max} = 11478); (H_2O) λ_{max} = 279 nm (ε_{max} = 10066); (0.01 M NaOH) λ_{max} = 272 nm (ε_{max} = 7846).

1-(2-{[Bis(phosphono)methoxy]methyl}ethyl)uracil (**34**). Material: 0.50 mmol of **33a**, 3.5 ml of TMSBr, 15 ml of CH_3CN. Hygroscopic yellow solid (yield 63%). For $C_{10}H_{18}N_2O_{10}P_2 \cdot 0.5H_2O$ (397.2) calculated: 30.24% C, 4.82% H, 7.05% N, 15.60% P; found: 30.37% C, 4.95% H, 7.19% N, 15.73% P. FAB MS: 389.3 (MH^+) (90). ^1H NMR (500 MHz, D_2O): 7.67 (d, 1 H,

$J_{H\text{-}6,H\text{-}5}$ = 7.9, H-6); 5.83 (d, 1 H, $J_{H\text{-}5,H\text{-}6}$ = 7.8, H-5); 3.91 (d, 2 H, $J_{3',2'}$ = 7.1, H-3′); 3.75 (d, 4 H, $J_{H\text{-}C\text{-}P}$ = 8.7, OCH_2P); 3.67 (d, 4 H, $J_{1',2'}$ = 5.6, H-1′); 2.45 (m, 1 H, H-2′). ^{13}C NMR (125.7 MHz, D_2O): 167.59 (C-4); 153.11 (C-2); 148.68 (C-6); 102.00 (C-5); 71.89 (d, $J_{1',P}$ = 11.9, C-1′); 66.67 (d, $J_{C\text{-}P}$ = 159.5, OCH_2P); 48.82 (C-3′); 38.70 (C-2′). UV: (0.01 M HCl) λ_{max} = 264 nm (ε_{max} = 6682); (H_2O) λ_{max} = 265 nm (ε_{max} = 8416); (0.01 M NaOH) λ_{max} = 264 nm (ε_{max} = 8516).

1-(2-{[Bis(phosphono)methoxy]methyl}ethyl)thymine (**37**). Material: 0.66 mmol of **36**, 3.5 ml of TMSBr, 15 ml of CH_3CN. Hygroscopic white solid (yield 78%). For $C_{11}H_{20}N_2O_{10}P_2 \cdot 0.5H_2O$ (411.2) calculated: 32.13% C, 5.15% H, 6.81% N, 15.06% P; found: 32.27% C, 5.27% H, 6.89% N, 15.22% P. FAB MS: 403.3 (MH^+) (45). ^1H NMR (500 MHz, D_2O): 7.52 (q, 1 H, $J_{H\text{-}6,CH3}$ = 1.2, H-6); 3.87 (d, 2 H, $J_{3',2'}$ = 7.2, H-3′); 3.75 (d, 4 H, $J_{H\text{-}C\text{-}P}$ = 8.7, OCH_2P); 3.66 (d, 4 H, $J_{1',2'}$ = 5.6, H-1′); 2.44 (m, 1 H, H-2′); 1.89 (d, 3 H, $J_{CH3,H\text{-}6}$ = 1.2, CH_3). ^{13}C NMR (125.7 MHz, D_2O): 167.82 (C-4); 153.18 (C-2); 144.46 (C-6); 111.31 (C-5); 71.91 (d, $J_{1',P}$ = 12.1, C-1′); 66.63 (d, $J_{C\text{-}P}$ = 159.7, OCH_2P); 48.55 (C-3′); 38.74 (C-2′); 12.00 (CH_3). UV: (0.01 M HCl) λ_{max} = 270 nm (ε_{max} = 8660); (H_2O) λ_{max} = 270 nm (ε_{max} = 8846); (0.01 M NaOH) λ_{max} = 269 nm (ε_{max} = 6760).

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